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Published in:
Animal Production Science

DOI:
10.1071/AN14171

Print publication: 01/01/2014

Document Version
Peer reviewed version

Nitrogen partitioning, energy use efficiency and isotopic fractionation measurements from cows differing in genetic merit fed low quality pasture in late lactation

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No information is available regarding nitrogen and energy use efficiency in relation to nitrogen isotopic fractionation measured from cows with different genetic merit. Eight high and eight low breeding worth cows were used to conduct a nitrogen balance study, and the results showed nitrogen isotopic fractionation and breeding worth were useful indicators of dry matter intake and nitrogen use efficiency of individual cows, respectively. Both indicators are easy to obtain on farm and could be further developed for dairy cow breeding programmes.

Abstract. The study was carried out to evaluate energy and nitrogen (N) use efficiencies of high and low breeding worth (BW) cow groups relative to N isotopic fractionation ($\Delta^{15}N$). Eight high and eight low BW cows (mean BW index = 198 and 57, respectively) in late lactation were used to conduct an N balance study with all cows fed autumn pasture. Individual cow pasture DM intake, N intake and N outputs of milk, urine and faeces were quantified. Plasma sample from each cow was harvested. Feed, plasma, faeces, urine and milk samples were measured for $\delta^{15}N$ and calculated for $\Delta^{15}N$. Urea nitrogen in milk and plasma, and urinary excretion of purine derivatives were also measured. The metabolisable energy (ME) intake, milk energy output, and energy and N use efficiencies of high BW cows were greater on average than low BW cows. Conversely, the ratios of urinary N excretion to faecal N excretion and urinary N excretion to N intake were greater for low BW cows than high BW cows. There was no effect of BW groups on manure N output, apparent N digestibility, retained N, purine derivatives excretion or ratio of purine derivatives excretion to ME intake. No relationships were found between N and energy efficiencies and $\delta^{15}N$ measurements. Regression analysis with individual cow measurement showed plasma $\delta^{15}N$ - feed $\delta^{15}N$ was negatively correlated with DM intake. N use efficiency was positively correlated with BW. High genetic merit cows are more efficient in N and energy use than lower genetic merit cows when fed low quality pasture in late lactation. Plasma $\delta^{15}N$ - feed $\delta^{15}N$ was proved to be a potential indicator of DM intake for individual cows when identical feed was offered. BW may be used to predict N use efficiency for individual cows.

Keywords: sustainability, isotopic discrimination, microbial protein synthesis, microbial energetic efficiency, manure nitrogen

Introduction

The breeding objective of New Zealand dairy industry is to produce cows that are more efficient at converting feed into profit. The genetic merit of a New Zealand dairy cow is measured by her breeding worth (BW) index, which ranks a cow on her expected genetic ability to breed profitable

and efficient replacements. The BW ranking is derived from breeding values which are based on ancestry, lactation performance and progeny information for seven traits (milk protein, milk fat, milk yield, somatic cell, live weight, fertility, and residual survival – a measurement of longevity) and are combined with specific economic values to derive a BW. In general, the BW system emphasises milk fat and protein production with a negative weighting applied to milk volume (Berry *et al*. 2007).

Earlier research demonstrated that high genetic merit cows produce more milk than low genetic merit cows when they are offered the same feed (Grainger *et al*. 1985; Coleman *et al*. 2010). However, limited information is available regarding nitrogen (N) partitioning and nutrient use efficiency of cows selected under the New Zealand BW system when fed pasture as a sole diet.

Previous research showed that N isotopic fractionation ($\delta^{15}N$) between plasma, milk and feed can be used to reflect N use efficiency of cows fed on different protein sources (Cheng *et al*. 2013). Further, plasma $\delta^{15}N$ - feed $\delta^{15}N$ is also a promising predictor of feed conversion efficiency in growing beef cattle reared on identical diets (Wheadon *et al*. 2014).

The objectives of this study were to 1. Compare energy and N use efficiencies of high and low BW cows; and 2. Investigate the use of $\Delta^{15}N$ as a simple indicator of energy and N use efficiency.

Materials and methods

Design and management

The study was undertaken at DairyNZ Lye Farm, Hamilton, New Zealand under the authority of the Ruakura Animal Ethics Committee. Sixteen multiparous Holstein-Friesian dairy cows in late lactation (221 ± 22.0 days in milk and 549 ± 50.3 kg live weight (LW), mean ± SD) were used, including eight high BW (H, mean BW index = 198) and eight low BW (L, mean BW index = 57) cows. Cows grazed on pasture prior to the study, and were then housed individually in metabolism stalls for four days to adapt to the facilities prior to commencing a five-day N balance study. Cows were fed freshly cut perennial ryegrass-based pasture at 0840 and 1600 h daily to ensure daily refusals were ~10 % of

offered pasture (*ad libitum*). Live weight was recorded at the start and the end of the five-day measurement period.

Intake and pasture quality measurement

Intake was measured daily per cow by weighing offered and refused pasture. Pasture samples (1 kg fresh weight) were collected twice daily at feeding and oven-dried at 65°C before grinding through a 1.0-mm sieve (Christy Lab Mill; Suffolk; UK). Pasture samples were analysed by Near Infra-Red Spectroscopy (NIRS systems 6500; feed TECH; New Zealand) for predicted crude protein (CP), organic matter digestibility, acid detergent fibre, neutral detergent fibre, and soluble sugar and starch content. Metabolisable Energy (ME) content was derived from predicted organic matter digestibility on the basis of an *in vitro* cellulase digestibility assay (Roughan and Hollan 1977; Dowman and Collins 1982), calibrated against *in vivo* standards (Corson *et al*. 1999).

Milk and plasma sampling and analysis

Cows were milked twice daily at 0730 and 1530 h. Milk production was recorded (Tru-test milk meters; Tru-test Ltd; New Zealand), and sub-sampled for composition analysis. A 10 ml blood sample was collected using a Li-heparinized evacuated tube from the jugular vein of each cow at 1130 h daily. Plasma was harvested following centrifugation at 1200 × *g* for 12 min at 4°C. At the end of the collection period, milk and plasma samples were pooled per cow and stored at -20°C until analyses were conducted.

Milk samples were analysed for fat, protein, and urea N (MUN) concentrations using Fourier-Transform Infra-Red Spectroscopy (Fossomatic™; Foss Electric; Hillerød; Denmark). Milk N content was derived from milk protein content divided by 6.38. Milk energy output (MJ/d) was calculated according to Rattray *et al*. (2007): [1.1 × milk production × (0.376 × milk fat % + 0.209 × milk protein % + 0.976)]. Plasma urea N (PUN) was quantified using a kinetic UV and colorimetric assay (Modular P800; Germany). Freeze-dried samples of milk, plasma, and pasture were ground through a 1.0-mm

sieve and weighed before $\delta^{15}N$ analysis was conducted using an Isotope Ratio Mass Spectrometer (PDZ Europa Ltd; UK; Cheng *et al*. 2011).

Faeces and urine collection and nitrogen analysis

Total outputs of faeces and urine from each cow were collected daily using the externally applied separators described by Meier et al. (2008). Urine containers were sealed to minimise N volatilisation losses. Daily urine samples were collected at 0730 and 1930 h, and acidified to pH < 4 using hydrochloric acid to prevent N volatilisation before storage at -20°C. Faecal samples were freeze dried before measurement. Representative liquid urine and freeze dried faeces from five days bulked samples per cow were sub sampled and measured for N concentration using a Variomax CN Analyser (Elementar Analysensysteme GmbH; Hanau; Germany). Retained N was calculated from N intake (NI) and N outputs: NI - milk N (MN) - faecal N (FN) - urinary N (UN). Manure N output was calculated by adding UN and FN together. Apparent N digestibility was calculated: (NI - FN) ÷ NI. Urinary excretion of purine derivatives (PD) was analysed using HPLC (Agilent 1100 series; Germany) following the method described by George *et al*. (2006).

Efficiency calculations

Energy use efficiency (EUE) = milk energy output (MJ/day) ÷ [DMI (kg/day) × ME content of pasture (MJ/kg DM)]

N use efficiency (NUE) = MN (g/day) ÷ NI (g/day)

Microbial energetic efficiency (MEE) = PD (mmol/day) ÷ [DMI (kg/day) × ME content of pasture (MJ/kg DM)]

Statistical analysis

The Genstat statistical package (version 15.1) was used for general analysis of variance and linear regression analysis. The statistical model included the treatment effect of BW (H and L). Data from

127 the five measurement days were averaged for individual cows for each variable and means were
128 analysed using ANOVA. The significance of treatment effect was declared at $P < 0.05$.
129
130 **Results**
131 The quality of pasture (Table 1) offered to cows in this study was typical for the autumn season in the
132 Waikato region of New Zealand. The intakes of DM and ME were higher for H compared with L (Table
133 2). Cows in the H group had 23% higher milk energy output and 15% higher feed energy utilisation
134 for milk energy output than cows in L (Table 2). Energy intake explained 44% of the variation in milk
135 energy output in the current study. NI and MN were 388 and 85, and 360 and 66 g/d for H and L,
136 respectively. NUE was 22% higher for cows in H than cows in L. FN increased as NI increased (r^2 =
137 52.8, SE = 8.74, $P < 0.001$). In addition, UN: NI and UN: FN ratios were, respectively, 13% and 15%
138 lower for H compared to L. However, there was no difference in manure N output between
139 treatments (Table 2). The calculated apparent N digestibility did not differ between H (0.67 g/g) and L
140 (0.68 g/g). Estimated retained N was 20.2 g/cow.d and 12.1 g/cow.d for H and L respectively, with no
141 statistical difference determined.
142 **[Insert Table1 and 2 here]**
143 N isotopic fractionation and urea N content in milk and plasma did not differ between H and
144 L groups. No statistical difference was detected for PD and MEE (Table 2).
145 Using individual cow measurements, significant positive relationships were found between
146 MUN and PUN [PUN (mmol/l) = -0.36 + 0.73 × MUN (mmol/l); r^2 = 34.1, SE = 0.412, $P < 0.05$], and milk
147 $\delta^{15}N$ - feed $\delta^{15}N$ and plasma $\delta^{15}N$ - feed $\delta^{15}N$ [plasma $\delta^{15}N$ - feed $\delta^{15}N$ (‰) = 1.95 + 0.53 × milk $\delta^{15}N$ -
148 feed $\delta^{15}N$ (‰); and r^2 = 24.8, SE = 0.254, $P < 0.05$]. However, no relationship was found between
149 nutrient use efficiencies and urea N in plasma and milk, and N isotopic fractionation of milk, plasma,
150 urine and faeces. Although no correlation was found between individual cow measurements for
151 plasma $\delta^{15}N$ - feed $\delta^{15}N$ and NUE, and BW and DMI; plasma $\delta^{15}N$ - feed $\delta^{15}N$ was significantly
152 correlated with DMI (Fig 1b), and BW and NUE was also correlated (Fig 1a).

153 **[Insert Fig1 here]**

154

155 **Discussion**

156 *Intake and milk energy output*

157 The higher DMI and energy intake observed in H compared with L (Table 2) is consistent with
158 Coleman *et al*. (2010). Milk energy output proportionally increased as energy intake increased, this
159 indicates energy intake is one of the major drivers for milk energy output in cows differing in genetic
160 merit.

161

162 *Energy use efficiency*

163 There are five potential factors that might contribute to the difference in EUE between H and L
164 groups: 1) mobilisation of body reserves to support production; 2) preferential partitioning of ME
165 intake between milk and body tissue; 3) change in energy utilisation in the rumen; 4) change in the
166 efficiency of utilisation of ME for milk production (i.e. k_l); or 5) differences in maintenance ME
167 requirements (ME_m). The contribution from body reserves to production was not quantified in this
168 study, therefore this possibility cannot be excluded. However, that the cows were in late lactation
169 and were likely in positive energy balance (Bauman and Currie 1980) suggest that energy
170 mobilisation would have been minimal. The change of energy level in the body (i.e. loss or gain
171 energy) would be difficult to quantify during five day measurement period. However, Davey *et al*.
172 (1983) showed that high genetic merit cows were able to partition more energy intake to support
173 milk production, and this may be linked with growth hormone and blood metabolite (e.g. glucose)
174 differences between the cows. Rumen function was indicated by calculated PD and MEE; both
175 showed no difference between groups (Table 2). Ferris *et al.* (1999) reported that k_l was not affected
176 by cow genotype. Therefore, a value of k_l = 0.6 was adopted to calculate ME_m, but no statistical
177 difference was detected.

178

Nitrogen use efficiency

Because FN increased in proportion to NI in this study, there was no difference between the H and L groups in apparent N digestibility. Similar to Ferris *et al*. (1999), the proportion of NI partitioned to urine (UN : NI) was lower for cows in the H group than in the L group (Table 2). The lower UN : FN ratio for H was due to lower UN and higher FN without altering rumen function in the current study. This is in contrast to previous report from Miller *et al*. (2001), who found the change of UN : FN was due to the change of rumen function. The partitioning of surplus N away from urine to faeces may contribute to reduce nitrate leaching to ground water and nitrous oxide and ammonia emission to the atmosphere (Varel *et al*. 1999). Higher NUE for H compared with L was reported by Ferris *et al*. (1999) and Wheadon *et al*. (2013) when comparing cow differing in genetic merit in Northern Ireland and New Zealand, respectively. The change in NUE in the current study was mainly achieved by 29% higher MN in H than in L, rather than altering rumen microbial protein synthesis or deamination in the liver (indicated by N isotopic fractionation) per se, which was suggested by Cheng *et al*. (2013). It is important to note that Wheadon *et al*. (2013) also suggested that higher metabolisable protein efficiency (i.e. milk protein output: metabolisable protein intake) for high genetic merit cows may be a factor that contributes to a higher NUE.

Relationships between urea and nitrogen use efficiency

The positive linear relationship between MUN and PUN detected in the current study is consistent with previous research (Hof *et al*. 1997; Kauffman and St-Pierre 2001). This relationship is due to urea produced by the liver and carried by the blood to the kidney. Urea can freely diffuse from blood to other body fluids, including milk (Kauffman and St-Pierre 2001). Although urea N in milk and plasma were found to be good indicators of NUE when various feeds were offered (Broderick and Clayton 1997), no relationship was found in the current study. The reason for this lack of relationship may be related to little difference in rumen fermentation, as previous research suggested that 80% of the variation in MUN is from rumen fermentation (Hof *et al*. 1997).

205

206 *Nitrogen isotopic fractionation in relation to nitrogen use efficiency and intake*

207 The levels of N isotopic fractionation presented in Table 2 are within the normal ranges previously
208 reported (Cheng *et al*. 2011; Wheadon *et al*. 2014). The weak but significant relationship between
209 milk $\delta^{15}N$ - feed $\delta^{15}N$ and plasma $\delta^{15}N$ - feed $\delta^{15}N$ is in agreement with Cheng *et al*. (2013). The effect
210 may be related to N isotopic fractionation during digestion and absorption or a common effect of the
211 endogenous contribution to the N sinks in the body.

212 In contrast to Cheng *et al*. (2013), there was no significant relationship between plasma $\Delta^{15}N$
213 (plasma $\delta^{15}N$ - feed $\delta^{15}N$) and NUE in this study. The absence of any difference in PD and MEE
214 suggests rumen function was similar for cows in both groups. Therefore the higher milk protein
215 synthesis observed for cows in the H group was probably due to improved utilisation of un-
216 degradable dietary protein rather than alterations in the supply of rumen degradable protein. In
217 addition, it is important to note that the predicted values of plasma $\Delta^{15}N$ from NUE using the
218 equation from Cheng *et al*. (2013) were 3.53 and 3.99 for H and L, respectively. In comparisons to the
219 measured values for plasma $\Delta^{15}N$ of 3.81 and 4.02 for H and L (Table 2), the predicted values were
220 within typical levels of standard deviation for isotope measurement (i.e. 0.3‰). Therefore, the
221 absence of a relationship between plasma $\Delta^{15}N$ and NUE in this study may also be due to inability of
222 Isotope Ratio Mass Spectrometry to detect isotopic fractionation differences less than 0.3‰.

223 It is important to note that considerable variation exists in pasture $\delta^{15}N$ from the same farm,
224 partly due to the relative contribution of clover and grass to the mix pasture sward (Cheng,
225 unpublished data). Legumes fix $\delta^{15}N$ depleted nitrogen from the atmosphere and generally have a
226 lower $\delta^{15}N$ than grass (Steele and Daniel, 1978). Therefore, the pasture $\delta^{15}N$ each cow group
227 ingested may have differed. Although all cows were offered with same swards, it is possible that feed
228 $\delta^{15}N$ varied as a result of differences in feed selectivity between H and L cows. Future study is needed
229 to sample feed from each cow or each treatment group in order to provide more accurate data to
230 establish correlations.

The negative relationship between DMI and plasma $\delta^{15}N$ - feed $\delta^{15}N$ (Fig 1b) was similar to the result presented by Sick *et al.* (1997), who showed when N is lower than the requirement for optimal growth of rats, transamination/deamination reactions drive a negative relationship between NI and plasma $\delta^{15}N$ - feed $\delta^{15}N$. In the current study, the feed contained only 2.43% N which is less than the requirement (i.e. 2.88% N) suggested for lactating cows by Pacheco and Waghorn (2008). Kristensen *et al.* (2010) showed that the higher the NI, the lower the absolute amount of recycled urea that would end up in the rumen. This should result in an enrichment of $\delta^{15}N$ in microbial protein which contributes to the plasma protein pool (Wattiaux and Reed, 1995), and consequently lead to a positive relationship between DMI and plasma $\delta^{15}N$ - feed $\delta^{15}N$. However, the contribution of rumen N isotopic fractionation in this study would have been minimal, since no variation was found for rumen function indicators (PD and MEE; Table 2).

Breeding worth in relation to nitrogen use efficiency and intake

The positive relationship found between NUE and BW (Fig 1a) indicates the current breeding objectives for dairy cows (which place a high weighting on milk protein and fat production) will also result in an improvement in NUE. This study also supports previous research showing that higher BW cows have higher intakes (Table 2; Grainger *et al*. 1985).

Conclusion

Selection of cows for increased genetic merit for milk fat and protein production (as indicated by the BW index) also leads to improved energy and N use efficiencies. Cows in the H group excreted a lower proportion of their N intake in the urine than cows in the L group. Milk and plasma urea N were not associated with NUE. In addition, this study demonstrated that N isotopic fractionation between plasma and feed may be developed as an indicator of DMI for individual cows when identical feed is offered.

Acknowledgements

This study was funded by the New Zealand Foundation for Research, Science and Technology (now Ministry of Business, Innovation, and Employment) and New Zealand dairy farmers through DairyNZ Inc. Thanks also go to DairyNZ Lye Farm staff and technicians and Lincoln University Analytical Laboratory technicians. The advice from Prof. John Roche (DairyNZ, New Zealand), Assistant. Prof. Yingluck Moonsan (Pibulsongkram Rajabhat University, Thailand), Dr. Alastair Nicol, Dr. Racheal Bryant, and Prof. Jon Hickford (Lincoln University, New Zealand) is appreciated.

References

Bauman AC, Currie WB (1980) Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. *Journal of Dairy Science* **63**, 1514-1529.

Berry DP, Shalloo L, Cromie AR, Veerkamp RF, Dillon P, Am PR, Kearney JF, Evans RD, Wickham B (2007) The economic breeding index: A generation on. In 'Technical report to the Irish Cattle Breeding Federation'. Ireland.

Broderick GA, Clayton MK (1997) A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *Journal of Dairy Science* **80**, 2964-2971.

Cheng L, Kim EJ, Merry RJ, Dewhurst RJ (2011) Nitrogen partitioning and isotopic fractionation in dairy cows consuming diets based on a range of contrasting forages. *Journal of Dairy Science* **94**, 2031-2041. doi: 10.3168/jds.2010-3763.

Cheng L, Sheahan AJ, Gibbs SJ, Rius AG, Kay JK, Meier S, Edwards GR, Dewhurst RJ, Roche JR (2013) Technical Note: Nitrogen isotopic fractionation can be used to predict nitrogen-use efficiency in dairy cows fed temperate pasture. *Journal of Animal Science* **91**, 5785-5788. doi: 10.2527/jas.2012-5378

Coleman J, Pierce KM, Berry DP, Brennan A, Horan B (2010) Increasing milk solids production across lactation through genetic selection and intensive pasture-based feed system. *Journal of Dairy Science* **93**, 4302-4317. doi: 10.3168/jds.2009-2591

283 Corson DG, Waghorn GC, Ulyatt MJ, Lee J (1999) Forage analysis and livestock feeding. In
284 'Proceedings of the New Zealand Grassland Association'. 61, 127-132.
285 Davey AW, Grainger FC, Mackenzie DDS, Flux DS, Wilson GF, Brookes IM, Holmes CW (1983)
286 Nutritional and physiological studies of differences between Friesian cows of high or low genetic
287 merit. In 'Proceedings of the New Zealand Society of Animal Production'. 43, 67-70.
288 Dowman M, Collins F (1982) The use of enzymes to predict the digestibility of animal feeds. *Journal*
289 *of the Science Food and Agriculture* **33**, 689-696. doi: 10.1002/jsfa.2740330802
290 Ferris CP, Gordon FJ, Patterson DC, Porter MG, Yan T (1999) The effect of genetic merit and
291 concentrate proportion in the diet on nutrient utilization by lactating dairy cows. *The Journal of*
292 *Agricultural Science*, *Cambridge* **132**, 483-490.
293 George SK, Dipu MT, Mehra UR, Singh P, Verma AK, Rangaokar JS (2006) Improved HPLC method for
294 the simultaneous determination of allantoin, uric acid and creatinine in cattle urine. *Journal of*
295 *Chromatography* **832**, 134-137.
296 Grainger C, Davey AWF, Holmes CW (1985) Performance of Friesian cows with high and low breeding
297 indexes 1. Stall feeding and grazing experiments and performance during the whole lactation.
298 *Animal Production* **40**, 379-388. doi: 10.1017/S0003356100040101
299 Hof G, Vervoorn MD, Lenaers PJ, Tamminga S (1997) Milk urea nitrogen as a tool to monitor the
300 protein nutrition of dairy cows. *Journal of Dairy Science* **80**, 3333-3340.
301 Kauffman AJ, St-Pierre NR. (2001) The relationship of milk urea nitrogen to urine nitrogen excretion
302 in Holstein and Jersey cows. *Journal of Dairy Science* **84**, 2284-2294.
303 Kristensen NB, Storm AC, Larsen M (2010) Effect of dietary nitrogen content and intravenous urea
304 infusion on ruminal and portal-drained visceral extraction of arterial urea in lactating Holstein
305 cows. *Journal of Dairy Science* **93**, 2670-2683. doi: 10.3168/jds.2010-3067
306 Meier S, Gore PJS, Barnett CME, Cursons RT, Phipps DE, Watkins KA, Verkerk GA (2008) Metabolic
307 adaptations associated with irreversible glucose loss are different to those observed during
308 under-nutrition. *Domestic Animal Endocrinology* **34**, 269-277.

309 Miller LA, Moorby JM, Davies DR, Humphreys MO, Scollan ND, MacRae JC (2001) Increased
310 concentration of water soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): Milk
311 production from late-lactation dairy cows. *Grass and Forage Science* **56**, 383-394.
312 doi: 10.1046/j.1365-2494.2001.00288.x
313 Nicol AM, Brookes IM (2007) The metabolisable energy requirements of grazing livestock. In 'pasture
314 and supplements for grazing animals'. (Eds PV Rattray, IM Brookes, AM Nicol) pp. 151-172. (New
315 Zealand Society of Animal Production Occational Publication No.14: New Zealand)
316 Pacheco D, Waghorn GC (2008) Dietary nitrogen – definitions, digestion, excretion and consequences
317 of excess for grazing ruminants. In 'Proceedings of the New Zealand Grassland Association'. 70,
318 107-116.
319 Roughan GP, Holland R. (1977). Predicting in-vivo digestibilities of herbage by exhaustive enzymatic
320 hydrolysis of cell walls. *Journal of the Science Food and Agriculture* **28**, 1057-1064.
321 Sick H, Roos N, Saggau E, Haas K, Meyn V, Walch B, Trugo N (1997) Amino acid utilisation and isotope
322 discrimination of amino nitrogen in nitrogen metabolism in rat liver *in vivo*. *Zeitschrift für*
323 *Ernahrungswissenschaft* **36**, 340-346.
324 Steele KW, Daniel RM (1978) Fractionation of nitrogen isotopes by animals: further complication to
325 the use of variations in the natural abundance of 15N for tracer studies. *Journal of Agricultural*
326 *Science* **90**, 7-9. doi: 10.1017/S002185960004853X
327 Varel VH, Nienaber JA, Freetly HC (1999) Conservation of nitrogen in cattle feedlot waste with urease
328 inhibitors. *Journal of Animal Science* **77**, 1162-1168.
329 Wattiaux MA, Reed JD (1995) Fractionation of nitrogen isotopes by mixed ruminal bacteria. *Journal*
330 *of Animal Science* **73**, 257-266.
331 Wheadon NW, Cheng L, Dewhurst RJ, Edwards GR (2013) Brief communication: Estimation of protein
332 utilisation efficiency and metabolisable protein efficiency in lactating cows groups by breeding
333 worth. In 'Proceedings of the New Zealand Society of Animal Production'. 73, 100-102.

334 Wheadon NM, McGee M, Edwards GR, Dewhurst RJ (2014) Plasma nitrogen isotopic fractionation
335 and feed efficiency in growing beef heifers. *British Journal of Nutrition* **6**, 1-7. Available at
336 http://www.ncbi.nlm.nih.gov/pubmed/24387820

337 **Table 1. Chemical composition and $\delta^{15}N$ content of feed**

338

Item	Pasture
DM (%)	21.0
Metabolisable energy (MJ/kg DM)	9.87
Crude protein (% on DM basis)	15.2
Neutral detergent fibre (% on DM basis)	58.9
Acid detergent fibre (% on DM basis)	31.8
Soluble sugars and starch (% on DM basis)	6.01
Feed $\delta^{15}N$ (‰)	2.97

339

340

Table 2. Genetic merit, live weight, intake, milk composition, energy use efficiency, nitrogen (N) partitioning, N isotopic fractionation and biomarkers of high (H) and low (L) breeding worth cows.

Item	H	L	SED	Significance
Genetic merit (breeding worth; $)	198	57	9.8	-
Live weight change (kg/day)	1.0	0.6	1.14	NS
Dry matter intake (kg DM/cow.day)	16.0	14.8	0.48	*
Milk production (kg/cow.d)	13.6	12.4	0.49	*
Milk fat (%)	5.72	4.98	0.347	NS
Milk protein (%)	3.98	3.42	0.124	***
Energy intake (MJ ME/cow.day) [A]	157.8	146.4	4.76	*
Milk energy output (MJ/cow.day) [B]	59.1	48.3	2.14	***
Energy use efficiency (MJ/MJ) [C]	0.38	0.33	0.013	**
N intake (g/cow.day)	387.9	359.7	11.72	*
Milk N (g/cow.day)	84.8	65.9	3.03	***
Faecal N (g/cow.day)	129.1	116.7	5.69	*
Urinary N (g/cow.day)	153.7	165.0	8.08	NS
Retained N (g/cow.day) [D]	20.2	12.1	9.00	NS
N use efficiency (g/g) [E]	0.22	0.18	0.009	**
Urinary N: N intake (g/g)	0.40	0.46	0.019	**
Urinary N : faecal N (g/g)	1.20	1.42	0.074	**
Manure N output (g/g) [F]	282.9	281.7	11.11	NS
Milk $\delta^{15}N$ - feed $\delta^{15}N$ (‰)	3.61	3.83	0.140	NS
Plasma $\delta^{15}N$ - feed $\delta^{15}N$ (‰)	3.81	4.02	0.137	NS

Urine $\delta^{15}N$ - feed $\delta^{15}N$ (‰)	-2.09	-1.93	0.231	NS
Faeces $\delta^{15}N$ - feed $\delta^{15}N$ (‰)	1.48	1.66	0.309	NS
Milk urea N (mmol/l)	6.88	7.19	0.250	NS
Plasma urea N (mmol/l)	4.62	4.94	0.297	NS
Purine derivatives (mmol/cow.day) [G]	148.6	160.5	11.63	NS
Purine derivatives : ME intake (mmol/MJ) [H]	0.94	1.11	0.077	NS

[A] dry matter intake × pasture metabolisable energy content

[B] [1.1 × milk production × (0.376 × milk fat % + 0.209 × milk protein % + 0.976)] according to Rattray *et al*. (2007)

[C] milk energy output : ME intake

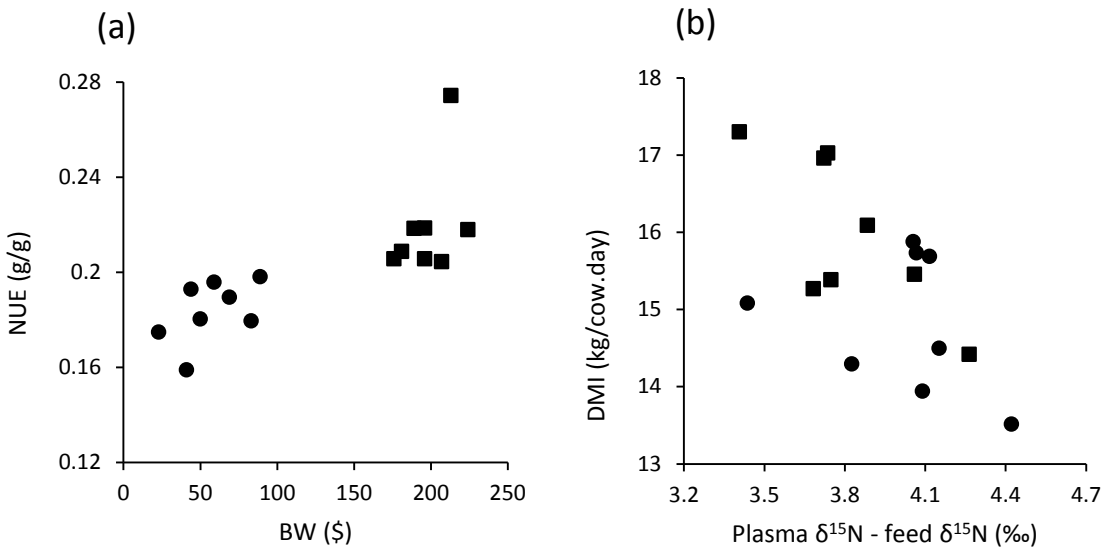
[D] N intake - faecal N - urinary N - milk N

[E] milk N : N intake

[F] urinary N + faecal N

[G] indicator of microbial protein synthesis

[H] indicator of microbial energetic efficiency



386

387 **Fig. 1.** Relationship between breeding worth (BW) and nitrogen use efficiency (NUE) (a), plasma $\delta^{15}N$
388 - feed $\delta^{15}N$ and dry mater intake (DMI) (b) of high (■) and low (●) genetic merit cows fed on autumn
389 pasture in late lactation.
390
391 Fig 1a: NUE (g/g) = 0.17 + 0.0003 × BW ($)
392 (n = 16, r^2 = 55.4, SE = 0.017, $P < 0.001$)
393 Fig 1b : DMI (kg/cow.d) = 24.7 - 2.37 × plasma $\delta^{15}N$ - feed $\delta^{15}N$ (‰)
394 (n = 16, r^2 = 32.5, SE = 0.909, $P < 0.05$)
395